

THE CHROMOSOMES AND RELATIONSHIP OF
CREPIS SYRIACA (BORNH.)

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INTRODUCTION

ALTHOUGH it has long been accepted that chromosome numbers are highly constant within species, a great many occurrences of deviation have been cited. It is very rarely, however, that a species is found in which variation is the rule rather than the exception. Such a discovery was reported by Hollingshead and Babcock (1930) in *Crepis*. While examining and comparing the chromosomes of a large number of species within this genus, individual plants of *C. syriaca* were found to possess from ten to thirteen chromosomes in somatic cells. The extra chromosomes, where present, were morphologically similar to one another, but different from any members of the basic group of ten. This condition in a species in which chromosome morphology is so well marked made it most favorable material for the investigation of supernumerary chromosomes. Other particularly advantageous characteristics of the species are the relatively low chromosome number and the fertility of plants with variant genomes.

The taxonomic relationship of *C. syriaca* is also of interest, particularly with respect to *C. alpina* L., of which it was formerly held to be a variety. Both species are in the same section of the subgenus *Barkhausia*, being most closely related to *Crepis rubra* L. and *C. foetida* L. of the same section (Babcock and Lesley, 1926, figs. 1 and 2, and table 2; Babcock and Navashin, 1930). In general appearance they are similar, yet there are many constant differences which serve to distinguish them as species. The more outstanding differences are tabulated as follows:

<i>C. syriaca</i>	<i>C. alpina</i>
1. Habit low, spreading.	1. Habit tall, erect.
2. Herbage light green, lacking tomentum.	2. Herbage gray, tomentose.
3. Radical leaves oblanceolate, acute, dentate or runcinate-pinnatifid or pinnately parted, the segments acute, dentate.	3. Radical leaves obovate-oblong, obtuse, denticulate, sometimes with 3-4 irregular shallow lobes near apex.
4. Cauline leaves laciniate at base.	4. Cauline leaves entire or denticulate at base.

* *Crepis syriaca* (Bornm.) Babcock, MS.

C. syriaca—(Continued)

5. Heads nodding before anthesis.
6. Heads fully expanded in anthesis.
7. Ligules deep yellow.
8. Anther-tube appendages 0.7 mm. long, obtuse.
9. Achenes about 14 mm. long, not always sharply divided into two types.
10. Pappus 4.5–5.5 mm. long.
11. Plants flower about 108 days after planting.

C. alpina—(Continued)

5. Heads erect before anthesis.
6. Heads partly expanded in anthesis.
7. Ligules pale yellow.
8. Anther-tube appendages 0.4–0.5 mm. long, acute.
9. Achenes about 18 mm. long, of two distinct types, marginal and inner.
10. Pappus 6–7 mm. long.
11. Plants flower about 135 days after planting.

Although, in respect to their comparative morphology, there appears little doubt of the correctness of recognizing these plants as separate species, still their relationship is so close that a cytogenetical analysis is warranted. Individuals from the two species were therefore crossed and the later generations subjected to a cytological and less extensive genetical investigation.

These problems were suggested by Professor E. B. Babcock, to whom the writer is indebted for constant assistance and advice. It is also a pleasure to acknowledge the generous coöperation of Professor R. E. Clausen throughout the course of the work.

MATERIALS AND METHODS

The majority of the *Crepis syriaca* plants used were derived from seed from herbarium material (accession number 1923). The original specimens were collected by M. Chijik, in Galilee, in 1924. Several plants were grown from this source by the writer in 1929 and from a study of these and their progeny most of the results here presented were obtained. Later accessions which were also used are numbers 3106, 3132, 3158, 3159, and 3167 from northern Lebanon near Hasroun, and 3168 from Antilebanon, near Baalbek. Accessions of *C. alpina* used for comparison and for hybridizing are numbers 1640 from the Copenhagen Botanic Garden, 1641 from Tiflis (indigenous in the Caucasus region), 2769 from the Leningrad Botanic Garden, and 2783 from the Charkow Botanic Garden.

Some variation in external morphology occurs among these accessions of *C. alpina*, especially between individuals of the Tiflis type and those from localities in Asia Minor, Kurdistan, western Persia, Transcaucasia, and Crimea. The former plants are sufficiently different to be classed as a subspecies of *C. alpina*. In habit they are somewhat more inclined to branch from the base of the plant, a character which is even more noticeable in *C. syriaca*. The radical leaves are acute, rather than obtuse, and are not so gray as the typical representatives of the species. The involucral bracts are purple-tipped, a character which is transmitted to all

F₁ plants in crosses with *C. syriaca*. The corollas are a brighter yellow color and the flower heads are, in general, smaller with shorter ligules. The achenes are of about the same length as typical *C. alpina*, but the beak is longer, in this respect also resembling *C. syriaca*. As will be pointed out later, these characters are of interest in connection with the possible origin of the latter species.

Root-tips obtained from plants five to ten days after transplanting to 6-inch pots were fixed in chrom-acetic-formalin solutions (cf. Hollingshead and Babcock, 1930). They were imbedded in paraffin and cut 8 μ thick. Buds were fixed in acetic alcohol (2 parts absolute alcohol, 1 part glacial acetic acid) and were examined from 70 per cent alcohol in iron-aceto-carmin.

Figures were drawn with the aid of a camera lucida at a magnification of 3750, using a Zeiss 1.8 mm. n.a. 1.3 oil immersion objective and Zeiss 12x compensating ocular.

THE SOMATIC CHROMOSOMES OF CREPIS SYRIACA

Aside from the supernumerary units present in *C. syriaca*, the chromosomes of this species are virtually identical with those of *C. alpina* ($2n = 10$). This fact coupled with the instability of plants with higher numbers is very good evidence that 10 is the basic diploid complement of *C. syriaca*.

A preliminary survey of the chromosomes of *C. syriaca* was presented by Hollingshead and Babcock (1930). Since this study was made, however, new chromosome numbers have appeared in the progeny of self-fertilized lines. Plants derived from accession number 1923, which had fourteen chromosomes or less, show five pairs of chromosomes consistently (see fig. 1b) resembling those of *C. alpina* very closely. In addition, there is a characteristic supernumerary unit which is present one, two, three, or four times in 11-, 12-, 13-, and 14-chromosome plants respectively. This extra chromosome appears as a small unit, possessing a subterminal constriction and bearing a large satellite at the proximal end (fig. 1a-X). In plants with fifteen, sixteen, and eighteen chromosomes respectively, the increase seems to involve more than this X chromosome. For example, in fig. 2b, showing a complement of fifteen chromosomes, there appear to be two extra E chromosomes and three X's (cf. fig. 1a). This may be due to a nondisjunction or nonconjunction in members of the basic complement and hence may be of rare occurrence. The chromosome groups containing sixteen and eighteen chromosomes (fig. 2c and d) do not show all the X chromosomes as pictured in figure 1a. There is little doubt, however, that the extra members are of this same type. Throughout the investigation, it was found that the difficulty in obtaining clear plates was greater in forms with higher numbers.

The drawings shown here were made with the primary purpose of depicting the variation in number, since only composite representations could indicate the complete morphology of the various groups.

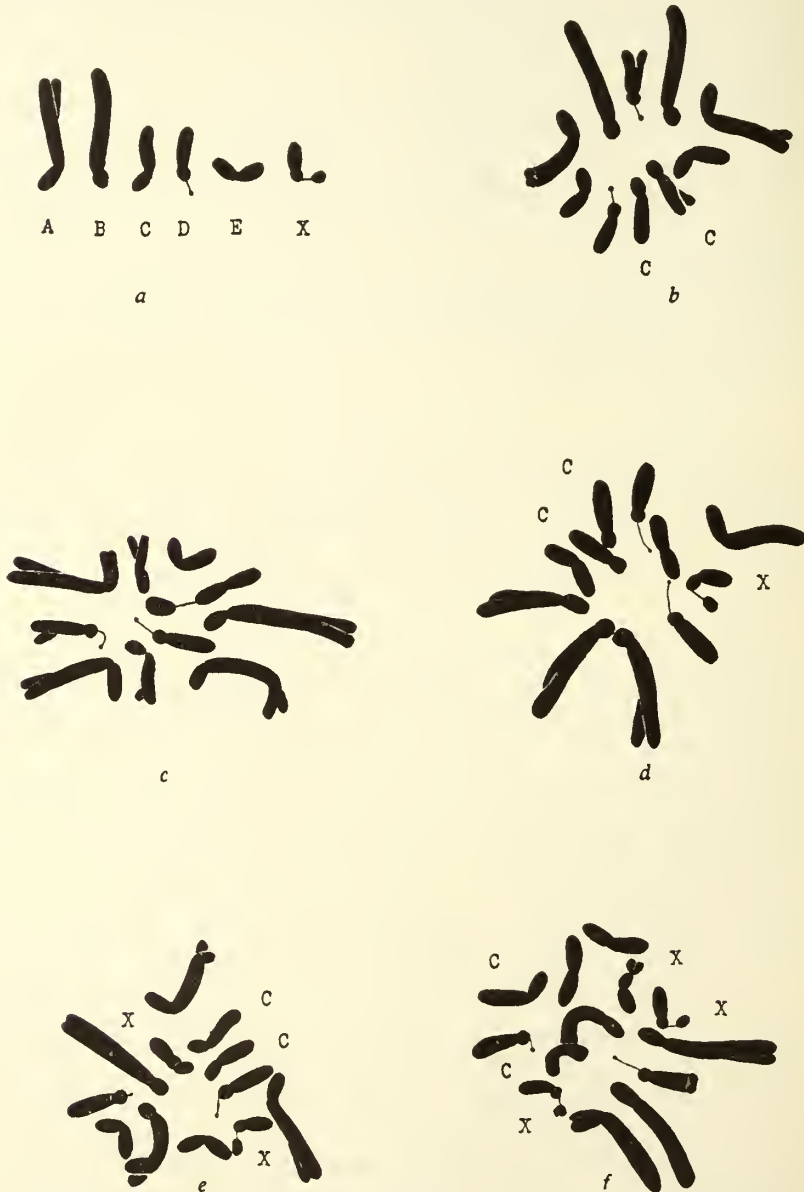


Fig. 1. *Crepis syriaca*. a, somatic chromosomes (haploid set); b, 1923 B6-2-8 (ten chromosomes); c, 3106 K1 (ten chromosomes)—note the large satellites on two non-homologous chromosomes; d, 1923 A 11-2-3 (eleven chromosomes), one supernumerary X chromosome; e, 1923 B7-1-3-1 (twelve chromosomes); f, 1923 B7-1-2 (thirteen chromosomes).



Fig. 2. *Crepis syriaca*. a, 1923 B7-3-1 (fourteen chromosomes); b, 1923 A 11-4-7 (fifteen chromosomes); c, 1923 A 11-4-6 (sixteen chromosomes); d, 1923 A 11-4-2 (eighteen chromosomes); e, 1923 A 11-4-6. Chromosomes of tetraploid cell, 32. The units in the lower right portion were in an adjacent section and some were obviously cut.

In the 16-chromosome plant obtained, a very high frequency of tetraploid cells occurred. The chromosomes of such a cell are shown in figure 2e. Those pictured at the lower right were from another section and some were obviously only a portion of chromosomes already drawn. A total of thirty-two units was counted in several cells and many others showed approximately the same number. Many binucleate cells were noted in this plant. Another unique circumstance was the presence of a large number of cells which exhibited profile views of metaphase chromosomes, with a consequent displacement of the usual regularity of cell layers. This was the only example of any abnormality in somatic mitosis which appeared during the study.

A much greater variability appeared in the cultures designated 3106. Figure 1c shows the chromosomes of a 10-chromosome plant from this accession. Instead of the usual five pairs ordinarily found in other 10-chromosome plants, a new abnormality appeared, involving the C and E chromosomes. Two large fragments of chromatin are attached to members of these pairs, upsetting the balance of the genom. All the plants derived from accession number 3106 appeared similar in their external morphology; yet there was not only a variation in chromosome number from plant to plant, but also variability in chromosome morphology within a single individual. So far no occurrences of variation in number of chromosomes among the cells of a single plant have been distinguished, nor do the two large pairs, A and B, ever become modified. In agreement with this observation Carothers (1917) suggests that, so far as she has observed, smaller chromosomes do not have a very pronounced effect when behaving irregularly, but that such irregularities associated with larger ones have a deleterious result. Among the varying chromosome groups in the cultures from accession number 3106, several cells were observed which contained a unit very similar in appearance to the X chromosome described earlier. It is conceivable that forms such as these may represent an intermediate step between normal 10-chromosome plants and the types described in the cultures grown from accession 1923.

VARIATION IN CHROMOSOME NUMBER

Nearly all the accessions that have been studied up to the present time have shown variation, although the amount of material available in some was quite limited. Plants grown from various accessions have been found to exhibit chromosome variability as follows: (a) variation in number between plants—1923; (b) variation in morphology within plants—3106, 3132, 3167, 3158; (c) variation in both number and morphology—3159; (d) a single plant of accession number 3168 showed the normal ten chromosomes of *C. syriaca*.

It is of course entirely possible that further variations in chromosome

number may be found in accessions other than 1923, since this one was studied much more intensively than the others.

In 1929 a number of cultures were grown from open-pollinated seed collected from plants grown from original accession number 1923. Table 1 shows the results of chromosome counts of plants obtained from this source. Among the cultures classified as derived from 10-chromosome parents, several were grown from seed collected from plants of which the chromosome number had not been determined. However, from the situation in the progeny there was no doubt concerning their chromosome complements.

TABLE 1
FREQUENCIES OF PLANTS HAVING DIFFERENT CHROMOSOME NUMBERS

Parental plants		Frequencies of plants obtained with numbers of:			
Number of plants	Number of chromosomes	10	11	12	13
7	10	81	2	6	
2	11	5	12	2	1
2	12	1		8	1
1	13			1	4

It is significant that, among the progeny, by far the greater number of plants had ten chromosomes. Since the parental plants were selected at random it is obvious that in the parental population 10-chromosome plants were more numerous than those with higher numbers. In each progeny group there was a marked tendency to reproduce the parental chromosome number. Among the progeny of 10-chromosome parents the variant numbers were 11 and 12; while the progeny of 11- and 12-chromosome parents varied in both directions from the parental number. In no plant was a complement of less than ten chromosomes found.

The foregoing results seem to indicate that certain lines have a greater tendency to vary than others. Thus, referring to table 2 of Hollingshead and Babcock (1930), the parent plant 1923-2 showed much more variation in its progeny than the plant or plants giving rise to culture 1923-A. From the results outlined here, it is altogether likely that plant 1923-2 had twelve chromosomes, while the parents of culture 1923-A had ten. Further similar evidence will appear later. Additional determinations of plants from accession number 1923 amplify the last line of this previously published table as follows:

	Chromosome number			
	10	11	12	13
27. 1923-2 Open-pollinated	2	3	8	5

In order to eliminate the possibility that fluctuations were brought about by crosses between plants which differ in chromosome number, individual selections were selfed to produce the later generations. Thus, except where specifically designated, the remaining data were obtained from self-fertilized lines.

TABLE 2
FREQUENCIES OF PLANTS (FROM SELFING) WITH DIFFERENT CHROMOSOME NUMBERS

Year grown	Pedigree	Chromosome number of parent	Frequencies of plants with numbers of:							
			10	11	12	13	14	15	16	18
1930	A2-10.....	10	2							
1930	A2-15.....	10	5							
1930	B6-2.....	10	8							
1931	A2-10-2.....	10	3							
1931	A2-15-5.....	10	9							
1931	B6-2-5.....	10	9							
1932	A2-10-1.....	10	2	1						
1932	A2-15-5-2.....	10	5							
1930	A11-2.....	11	1	5	4					
1931	A11-2-8.....	11		6	1	1				
1930	B7-3.....	12			3	1	5			
1931	A11-2-1.....	12	3		4	2	1			
1931	A11-2-6.....	12	3							
1931	B7-8.....	12	1		2					
1931	A11-4.....	13				2	3	1	2	1
1932	A11-4-3.....	13	2		1		1			
1932	A11-4-4.....	14		1						

It is unfortunate that larger numbers of plants could not be examined. This was often impossible because of the small amount of seed set by certain plants and the inviability of plants with particular somatic complements. In other examinations larger numbers would not have contributed any valuable information since the chromosome numbers could be predicted from previous determinations. In 1932 all the progeny from an 11-chromosome individual died after reaching the rosette stage. Only one survived until root-tips could be collected and these were so poor that an accurate determination was impossible. At the same time an entire culture of plants from a 10-chromosome parent succumbed. Apparently there was a factor influencing the physiology of these plants, making them more susceptible to slightly adverse environmental conditions. Other cultures, alternating with these, remained perfectly healthy.

Table 2 shows the frequencies of plants having different chromosome numbers in self-fertilized lines. In all the cultures derived from 10-chromosome parents, only one variant appeared. This 11-chromosome individual may have been the result of an accidental cross or of a mixture of seed, but at any rate a very high degree of constancy is revealed.

This result is only to be expected if ten is the "normal" somatic complement of the species. In the progenies obtained from open-pollinated plants, the majority of individuals have the same chromosome count as their parents. In selfed lines, however, this tendency grows progressively less marked with the increase in chromosome number. It is still quite noticeable in the 10- and 11-chromosome series, but in 12- and 13-chromosome lines the tendency is toward the production of still higher numbers. In the culture from an open-pollinated 13-chromosome plant, for example, four 13's and one 12 were obtained. When another 13-chromosome plant was selfed, individuals with thirteen, fourteen, fifteen, sixteen, and eighteen chromosomes were obtained. This, of course, may have been owing to differences in the parental plants. In general, there did not appear to be any tendency on the part of plants with larger numbers to return to the 10-chromosome type, although, when this condition was reached, it was virtually always maintained.

RELATION OF CHROMOSOME NUMBER TO EXTERNAL MORPHOLOGY

In respect to the external characters exhibited by the plant, the extra chromosomes appear to have small and inconsistent effects. In general, in plants having from two to eight supernumeraries, the flower heads are small and asymmetrical, and frequently have split ligules. The 10- and 11-chromosome individuals are usually normal in this respect although some individuals among the plants possessing eleven chromosomes have narrower ligules with longer teeth than in the normal forms (plate 12, figs. 2 and 3). There is also a tendency for plants with higher numbers to have broad, coarse leaves with margins which are merely toothed instead of margins more deeply cut, as in the 10-chromosome types. This alteration seems to depend more on the parental complement than on the number of chromosomes actually contained in plants with the type of foliage described. The difference is brought out clearly in the 1932 cultures. In the progeny of a 13-chromosome plant, two individuals appeared having ten somatic chromosomes. These were similar to normal individuals except in their broad radical leaves and generally more robust habit. There is here an indication that other changes besides gross morphological ones may be taking place in the chromosomes. As far as can be seen from a microscopic examination, the chromosomes appear identical in all 10-chromosome plants, regardless of their origins. On the whole, the 10-chromosome individuals are of a paler green color than are the plants containing supernumeraries, but there is sufficient variability to make a thorough color investigation of little value.

Another occasional abnormality is the development of an irregular rosette. This occurs in individual plants and is associated with eleven,

fourteen, and sixteen somatic chromosomes. In spite of their morphological irregularity, these plants show uniform reduction divisions and produce a high percentage of good pollen. No plants of this type occur in the lines characterized by the possession of ten chromosomes. In several of the irregular forms the rosette has two distinct centers of growth, giving to some of the leaves the appearance of being inverted.

Most of the cultures derived from 10-chromosome plants were consistently uniform in external morphology. However, in 1930 an interesting new type appeared among them. In two cultures, five small plants appeared, which were approximately 15 cm. high as compared with 45 cm. for their normal sibs. The contrast between these two types can readily be seen by a comparison of plate 12, figures 1 and 2. Little attention was given these plants at the time, since occasionally dwarfing occurs as a result of unfavorable growing conditions. In 1931, however, a culture of ten plants was grown from one of these variants, all of which were of the same dwarf habit. From another individual, in the same year, progeny were grown which showed segregation for this character. The tallest plant in the culture was 50 cm. in height, the smallest about 15 cm. The nine plants of this culture which reached maturity could readily be grouped into two size-classes of 7 tall : 2 dwarf. Although these numbers are far from adequate as statistical data, it seems altogether likely that the first selected plant referred to must have been homozygous, and the second, heterozygous for the character. Hence, it is probable that an intermediate height condition appears in the heterozygote, as it was a relatively small plant that gave rise to the segregating population. In 1932 a culture was grown from one of the dwarf segregants, of which all the plants were dwarf. In order to determine the nature of the genes associated with the new character, crosses were made between the two types. The resulting seeds were inviable.

MEIOSIS IN *CREPIS SYRIACA*

A study of microspore mother-cells in this genus is attended with more than ordinary difficulty. The chromosomes are very large and irregular in shape, making an analysis of the meiotic behavior not an easy matter. The majority of cells in smear preparations present a profile view of the metaphase plate and the total number of cells obtained from one floret is small.

The 10-chromosome plants showed five pairs at I M in all preparations where a definite determination was possible. In other *Crepis* species which have been examined, a small amount of variation has been seen, so that it is very likely that a similar condition would occur here if the studies were prolonged. In good preparations a large number of cells were seen which had the appearance of the one represented in figure 3a. Figure 3b

shows a drawing of a plate typical of the 11-chromosome forms. Six units were consistently present involving five bivalents and a univalent. Usually the bivalents behaved normally, although at times one of these units was seen to divide precociously. The univalent divided at the first division and at the second the halves separated to either pole. Thus, apparently 5- and 6-chromosome gametes were regularly produced by this type of plant. On this basis an 11-chromosome plant when selfed should yield 10- 11- and 12-chromosome progeny in the ratio of 1:2:1 if a similar situation exists in the embryo sac. Table 2 shows, however, that the distribution is not of this type.

The discrepancy between observed and expected frequencies of 10-, 11-, and 12-chromosome plants derived from 11-chromosome parents may result from a qualitative difference in the chromosomes involved. There seems to be a definite tendency for plants with more than five pairs of chromosomes to yield progeny with more supernumeraries than they possess. This fact leads to the suggestion that the supernumerary unit may possess genetic material which is of considerable value in development. However, 10-chromosome plants show little or no inclination toward yielding progeny with larger numbers of chromosomes. Let us assume that the X chromosome arose from a reorganization of the chromatin of the basic five pairs by fragmentation and attachment. Then the portion of genetic material contributed would be duplicated in the genomes of plants with more than ten chromosomes. If this portion were associated with increased vigor or viability, it might reasonably be expected that 6-chromosome gametes of an 11-chromosome plant would function at the expense of 5-chromosome gametes and hence would lead to a preponderance of higher-numbered forms.

In several preparations there was an aberrant behavior of the divided univalent at I A in 11-chromosome plants. Instead of the halves going to opposite poles, they were both seen in close proximity to one end of the cell even after the other chromosomes had merged and were no longer distinguishable as separate units (fig. 3e). No micronuclei were apparent, however, so these must have been incorporated in the daughter nuclei at the second division.

A greater amount of irregularity could be seen in 12- and 14-chromosome plants. In spite of this, a remarkably high percentage of complete pairing was evident. In 12-chromosome forms only minor irregularities were noted and these occurred infrequently. In nearly all cells which presented clear views, six bivalents could be distinguished (cf. fig. 3c). Occasionally a precocious division of a bivalent occurs, or lagging of the univalents after the bivalents have split.

In 14-chromosome plants (fig. 3d) a peculiar condition was revealed. Some flower heads showed a very high percentage of regularity in meiosis, seven bivalents being regularly formed. In other florets on the

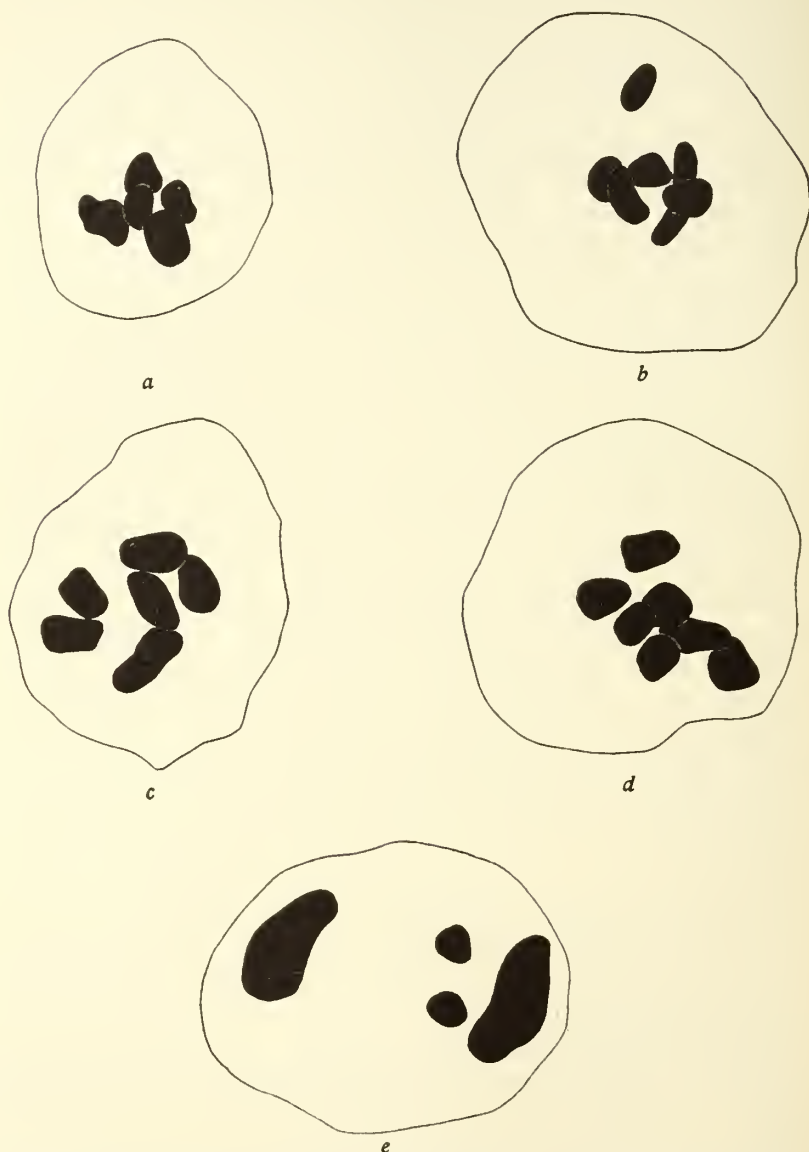


Fig. 3. *Crepis syriaca*. *a*, 1923 B6-2-5 (ten chromosomes forming five bivalents); *b*, 1923 A2-10-1-1 (eleven chromosomes forming five bivalents plus one univalent); *c*, 1923 A 11-2-1-7 (twelve chromosomes forming six bivalents); *d*, 1923 A 11-4-1 (fourteen chromosomes forming seven bivalents); *e*, 1923 A 11-4-4-1 (eleven chromosomes; telophase showing both halves of divided univalent at one pole).

same plant there was great irregularity. Chromatin masses appeared in place of the usual independent units. These in many preparations were accompanied by small fragments and division seemed to be brought about by a simple fission of the general mass. This highly abnormal type of division would account for the large percentage of poor pollen formed by such plants.

In maize, Randolph found all types of pairing among the extra chromosomes. An occurrence was also reported of pairing between one of the supernumeraries and one of the normal bivalents. There was a difference in the staining reaction of the extra univalents, the latter being somewhat darker than the rest of the complement.

EFFECT OF CHROMOSOME NUMBER ON POLLEN FORMATION AND FERTILITY

Although there appears to be no clear-cut correlation between chromosome number and morphology in *C. syriaca*, an excess of chromatin does have a marked effect on pollen formation and fertility. Furthermore, the amount of excess chromatin has a greater effect apparently than the chromosome unbalance. This can readily be seen by an examination of table 3, which shows the percentage of undeveloped pollen grains in different chromosome-number classes. Since some of the figures were obtained, it has been found that occasionally the first flower formed yields highly aberrant results. For this reason there have been omitted a few plants which were obviously of this type and which would consequently give misleading average values for the different classes.

The plant numbers marked with an asterisk refer either to individual plants or to an average of two, which occurred in populations derived from plants with chromosome numbers other than their own. The rest were derived from plants with the same number, as is indicated in the second column. Only a few 14-chromosome plants were available for pollen counts and these showed from 30 per cent to 50 per cent undeveloped grains.

These figures are not conclusive, since a comprehensive study of pollen development and fertility was not intended. The table does show, however, that there is a marked difference between 10-chromosome forms and those which contain larger chromosome complements. It might well be expected that 12-chromosome individuals, having a balanced chromosome condition, would show a higher percentage of good pollen than the 11's and 13's. This does not prove to be the fact. There seems to be a definite tendency for the higher-numbered individuals to have a larger percentage of undeveloped pollen, irrespective of whether the chromosome number is odd or even.

TABLE 3
CHROMOSOME NUMBERS AND POLLEN DEVELOPMENT AND FERTILITY

Pedigree number	Chromosome number	Percentage of poor pollen	Fertility
A2-10.....	10	1.9	Fair
A2-15.....	10	1.4	Poor
B6-2.....	10	2.9	Fair
A2-15-5.....	10	0.6	Good
B6-2-5.....	10	2.5	Fair
A2-15-5-2.....	10	1.3	Fair
A2-10-1.....	10	0.9	Good
*A11-2-5.....	10	4.2	Poor
*A11-2-1.....	10	4.5	Fair
*A11-2-6.....	10	2.1	Fair
*B7-8-2.....	10	0.7	Poor
*A11-4-3.....	10	18.7	Poor

AVERAGE PERCENTAGE OF POOR POLLEN FOR 10-CHROMOSOME PLANTS, 3.4

A11-2.....	11	14.5	Poor
A11-2-8.....	11	20.1	Poor
*A11-4-4.....	11	10.1	Fair
*A11-4-5.....	11	1.9	Fair
*A2-10-1-1.....	11	21.2	?

AVERAGE PERCENTAGE OF POOR POLLEN FOR 11-CHROMOSOME PLANTS, 13.5

B7-3.....	12	34.3	Poor
A11-2-1.....	12	22.1	Fair
B7-8.....	12	17.0	Fair
*A11-2.....	12	13.2	Poor
*B7-1-5.....	12	21.7	Poor
*A11-2-8-7.....	12	50.2	Poor
*B7-1-3-1.....	12	2.3	?
A11-4-3-3.....	12	10.9	?

AVERAGE PERCENTAGE OF POOR POLLEN FOR 12-CHROMOSOME PLANTS, 21.4

B7-1.....	13	27.2	Poor
A11-4.....	13	9.1	Fair
*B7-3-9.....	13	48.1	Poor
*A11-2-1-1.....	13	50.0	Poor

AVERAGE PERCENTAGE OF POOR POLLEN FOR 13-CHROMOSOME PLANTS, 33.6

* Either an individual plant or an average of two plants.

If the pollen grains containing supernumeraries are the nonfunctional ones, it is hard to see why the higher-numbered forms do not yield chiefly 10-chromosome plants. The explanation of the results obtained may lie in the possible occurrence of functional maternal gametes with more than five chromosomes or of nonfunctioning pollen grains having five chromosomes which lack something contained in the extra chromosome.

Little evidence of correlation between pollen development and fertility has been shown in the results obtained up to the present time. To be sure, the 10- and 11-chromosome plants show a higher degree of fertility and a lower percentage of deficient pollen in general. Among individual plants, however, those with the best pollen are not necessarily the most fertile. The 11-chromosome plants are virtually as fertile as those with ten, while 12's, 13's and 14's are nearly sterile and the rest completely so; and these do not show a proportional increase in nonfunctional pollen grains.

RESULTS OF CROSSES WITH *CREPIS ALPINA*

Following the method outlined by Hollingshead (1930), several crosses were made between individuals of *C. syriaca* from accession number 1923, and *C. alpina* plants obtained from accessions 2783, 1641, and 2769, in order to determine, as far as possible, cytogenetical relationships between the species.

When *C. syriaca* is used as the female parent there is a much higher percentage of seed set than in the reciprocal cross. This may be due to the fact that usually *syriaca* plants were chosen which had a chromosome number higher than ten; and it has been shown in wheat hybrids involving plants which differ in chromosome numbers, that it is preferable to use the plant with the higher number as female (Watkins, 1927). Only eleven of the achenes obtained from the cross *alpina* ♀ × *syriaca* ♂ produced plants and of these all but two died before reaching maturity. A glance at plate 13, figure 3, shows the total dissimilarity of these to the other hybrids and to the parents. The cause of this dissimilarity is as yet unknown. It is possible that environmental conditions may provide the true explanation, although occurrences of unlike reciprocal hybrids have been reported. Further crosses are planned in order to clear up this question.

From the reciprocal cross, twenty-six hybrids were obtained, being derived from *syriaca* plants having ten, eleven, twelve, and thirteen somatic chromosomes. In all of them hybrid vigor was marked. With respect to leaf type and general habit they were more like the *alpina* parent, but they had the flower-head type and color and the nodding buds of *syriaca*. Nearly all of them were virtually as early maturing as *syriaca* and were consistently highly fertile. The latter characteristic,

along with the compatibility of their chromosomes, is evidence of the close relationship of the two species.

In crosses where *alpina* was used as a female parent, the resulting hybrids all possessed ten chromosomes. This, of course, may only be because of the small number obtained. However, in view of the amount of variation ordinarily met with and the fact that only plants with supernumeraries were used as pollen parents, it is much more likely that there is a preferential functioning of pollen grains in favor of 5-chromosome grains.

In the *syriaca* ♀ × *alpina* ♂ crosses, the hybrids revealed varying numbers of chromosomes, the situation being similar to that in the female parent. The resulting chromosome numbers are shown in table 4. Since it may be assumed that the *alpina* parent always contributes five chromosomes, the number present in functioning egg-cells is readily calculated.

TABLE 4
FREQUENCIES OF HYBRIDS HAVING DIFFERENT CHROMOSOME NUMBERS

Parental chromosome numbers	10	11	12	13	Chromosome number in functioning egg-cells
10 x 10	13				5
11 x 10	1	2			5, 6
12 x 10	4	1	2	1	5, 6, 7, 8
13 x 10		2			6

As with *C. syriaca*, the F_1 progeny of 10-chromosome parents all possessed ten chromosomes. Where a higher number was involved, variation again occurred and to a considerable degree. The somatic complements of the hybrids resemble closely the ones which have been described in connection with different number-groups of *syriaca*. The basic five pairs are consistently present with one, two, or three of the small supernumeraries, the appearance of which has already been described. Assuming a regular reduction division, we should expect that the progeny of a 12- and a 10-chromosome plant would contain eleven chromosomes. Such, however, does not prove to be true. Not only are both the extra chromosomes present in the offspring, but even an additional one may be added to yield a 13-chromosome form. As has already been indicated by the production of an 18-chromosome *syriaca* individual from a 13-chromosome parent, there must be a further multiplication of extra chromosomes in the divisions prior to, or during, meiosis. If feasible, a study of embryo-sac mother-cells should be of great assistance in clearing up this point. It has already been pointed out that *syriaca* pollen probably does not transmit the extra chromosome in crosses with *C. alpina*. Furthermore, the entire lack of resemblance of these individuals to their maternal parents

and their similarity to one another precludes the assumption of a development initiated by a doubling of the maternal genom. It is possible that *syriaca* pollen may transmit the extra chromosomes in self-fertilization, while showing quite a different behavior in *alpina* pistils.

MEIOSIS IN THE F_1

An extensive investigation of meiotic behavior was not attempted since a preliminary survey indicated that this was not recognizably different from the parental type. This is only to be expected, considering the similarity between the chromosome groups of the two species involved and the high degree of fertility exhibited by the F_1 plants. In all the 10-chromosome F_1 's examined, complete pairing was consistently the rule.

POLLEN STUDIES IN THE F_1

Pollen counts of F_1 plants revealed some rather unexpected results. It is significant, for example, that in 10-chromosome plants the percentage of poor pollen was much greater than in *C. syriaca* itself while the percentage of fertility was as high, if not higher. This indicates that there is not a very close correlation between percentage of good pollen and degree of fertility. However, there is always considerable variation even within an individual plant from time to time, so that these results are not conclusive evidence on this point. The percentages of poor pollen and the parental chromosome numbers of the F_1 cultures are shown in table 5.

TABLE 5
PARENTAL CHROMOSOME NUMBER AND POLLEN DEVELOPMENT IN F_1

Culture number	Parental chromosome number	Average percentage of poor pollen
X	10 x 10	58.7
X3	10 x 10	34.6
X8	10 x 10	59.4
X2	11 x 10	41.1
X4	12 x 10	64.7
X6	13 x 10	90.1

The fact that in hybrids the percentage of undeveloped pollen was higher than in the parental species, irrespective of chromosome number, was of assistance in determining whether a hybrid had actually been obtained. Thus, a supposed hybrid which appeared very like *C. syriaca* in external morphology, was found to have only 1 per cent poor pollen and was for this reason classified as resulting from accidental self-fertilization.

The number of plants examined was of necessity very small, but, even so, it seems fair to draw some tentative conclusions from the examinations. The individual results are not tabulated because of the variation just referred to, but they indicate that the parental chromosome number had a greater influence on pollen production than the number actually contained in the individual. For example, two 11-chromosome plants in the progeny of a 13-chromosome *syriaca* parent had values of 89.1 per cent and 91.2 per cent poor pollen, while similar plants in the progeny of an 11-chromosome progenitor had only about 40 per cent of undeveloped grains.

As before, there is an increase in proportion of nonfunctioning grains with an increase in number of extra chromosomes. In spite of the unbalance in the 11-chromosome plants, they produce progeny which exhibit considerably less poor pollen than the 12's or 13's. This is especially surprising in view of the fact that six pairs are consistently found in 12-chromosome plants.

F₂ CHROMOSOME NUMBERS

From the results discussed so far it might reasonably be expected that in the F₂ progeny a variability in chromosome number similar to that in the parental and F₁ types would be expressed. In respect to the 10-chromosome lines this proved to be true, all individuals determined hav-

TABLE 6
FREQUENCIES OF F₂ PLANTS WITH DIFFERENT CHROMOSOME NUMBERS

Culture number	Parental number	Frequencies of plants with:					
		10	11	12	13	14	15
X3-2	10	10					
X4-1	10	9					
X2-2	11		1	9			
X6-1	11	1		8			
X7-1	12			1	1	2	1

ing ten somatic chromosomes. However, in the progeny of 11-chromosome F₁ individuals the majority of plants had twelve, while the self-pollination of a 12-chromosome type gave rise to considerable variation again, the tendency being toward still higher numbers. The obtaining of such a large proportion of 12-chromosome plants from an 11-chromosome parent is very difficult to explain. Referring to the 11-chromosome groups of tables 1 and 2, it can be seen that eighteen out of a total of twenty-eight had eleven chromosomes, while only three had twelve. In these F₂ cultures seventeen out of a total of nineteen had twelve, while only one had eleven (cf. table 6). This may indicate a tendency to pro-

duce a balanced number of chromosomes and suggests again the theory that the supernumeraries contain some material which is beneficial in development. When achenes from 12-chromosome F_1 plants were sown, however, the resulting plants, which are obtained only with difficulty, showed greater variation in chromosome numbers than the progeny of 11-chromosome individuals, in spite of the unbalance in the cells of the latter type. F_3 results should prove of great interest in connection with the stability of different chromosome groups. In table 6 are shown the frequencies of F_2 plants having various chromosome counts. These plants were all quite uniform and, as in F_1 , they resembled their *alpina* grandparents in habit, leaf type, and fertility, and showed segregation with respect to flower-head type. They did not mature quite so early as the F_1 , but much earlier than pure *alpina*.

DISCUSSION

Supernumerary chromosomes have been reported in *Zea* (Randolph, 1928), *Secale* (Gotol, 1924; Belling, 1925), *Ranunculus* (Langlet, 1927; Sorokin, 1927), *Oenothera* (Lutz, 1916), *Rosa* (Blackburn and Harrison, 1921), *Crepis* (Navashin, 1926), *Fritillaria* (Darlington, 1930), *Viola* (Clausen, 1931), and in a number of insects (Stevens, 1908; Carothers, 1917; Wilson, 1909). In most of these the extra chromosomes arise by (1) *duplication*, (2) *fragmentation*, or (3) *hybridization*.

Duplication of chromosomes by means of nondisjunction or nonconjunction was found by Navashin to be the cause of a small amount of spontaneous chromosomal alteration in *Crepis tectorum*. The supernumeraries in this species had a greater effect on the viability of the plants than those found in *C. syriaca*. The morphology of the extra unit in the latter species precludes the possibility of its origin by duplication although this may be a factor in the multiplication of it in higher-numbered plants.

Fragmentation of chromosomes of the normal complement is assumed to be the cause of the extra units in *Secale*, *Fritillaria*, and *Oenothera*. According to Geerts (1911) this phenomenon is likely to occur in extra chromosomes as a result of hybridization. Measurements of chromosome lengths in *C. syriaca* revealed that there was a definite linear increase of the chromosomes as a group where supernumeraries were present. Thus it is very unlikely that they arose by a simple fission of a member of the basic genom. It is possible that fragmentation following an attachment of two chromosomes may have led to the production of the X chromosomes of *C. syriaca*. Carothers (1917) explains in this way the origin of extra units in *Trimerotropis* and *Circotettix*.

Hybridization is the factor to which is ascribed the origin of extra chromosomes in *Ranunculus* and *Viola*. The fact that in *C. syriaca* varia-

bility is so widespread suggests that the species has arisen by hybridization (involving *C. alpina*, in all probability). So far, no material has been examined which did not show a fairly high proportion of individuals with extra chromosomes, although in all the material the chromosome number was constant for the individual. Furthermore, there seems to be no tendency toward reversion to the 10-chromosome type. Plants of this type are regularly found to be in the majority and, when selfed, yield only 10-chromosome progeny, yet accessions from the wild which were studied showed many extra-chromosome individuals.

The only related species which possesses a chromosome similar to the extra units found in *C. syriaca* is *C. rubra*. This species is indigenous in Greece and is not known from Syria, the home of *syriaca*, nor even from Asia Minor. If natural hybridization with *C. rubra* has brought about the increase in chromosome number, it must be assumed that the cross was effected a very long time ago and that the unbalanced condition has been maintained. Furthermore, a careful examination of external morphology fails to reveal the presence of any distinctive *rubra* characters in *syriaca*. For these reasons it is necessary to look further for the origin of the supernumeraries.

Although it appears unlikely that *C. rubra* was involved with *C. alpina* in the origin of *C. syriaca*, because of their wide geographic separation and the absence of any distinctive *rubra* characteristics in *syriaca* plants, there are other possible explanations involving natural hybridization. It has already been pointed out that two distinct strains of *C. alpina* are known which grow in fairly close proximity to each other, the typical form being distributed from Asia Minor to Transcaucasia, the other in the Caucasus region. Furthermore, the latter type possesses some characters which are more like those of *syriaca* than those of the typical form. It is quite possible that, after the former became distinct, natural crossing was effected between them, which, with later genic and chromosomal modifications, produced individuals of the *syriaca* type. These may have become isolated and gradually moved in a southerly direction to occupy the Lebanon region, while the typical form of *C. alpina* was restricted to the present more northern range and the other to the Caucasus and neighboring localities. This would explain the great similarity of the basic chromosome groups of all three races, as well as the origin of certain taxonomic characters of *C. syriaca*. For example, the flowers of the latter are brighter yellow than those of typical *C. alpina*, but are very similar in color to those of *alpina* plants from the Caucasus region. In habit and achene characters, also, *syriaca* and the Caucasian form of *alpina* show a tendency toward mutual resemblance. Such a hybridization might lead to an alteration in the normal chromosome behavior, giving rise to the variation characteristic of *C. syriaca*.

Another explanation of the origin of these forms is that *C. syriaca* and *C. alpina* (Caucasian type) are both the result of hybridization with some form now unknown. This is suggested by the fact that the Caucasian form is more or less intermediate between *C. syriaca* and typical *C. alpina*. This interpretation, however, is less satisfactory since it assumes the existence of an unknown form. Moreover, the present situation in respect to both comparative morphology and the chromosomes in the two forms of *C. alpina* and *syriaca* can be reasonably explained without recourse to this alternative hypothesis.

Navashin (1931a) has shown how chromosomes may become altered in nature. Plants of *C. tectorum* which were abnormal in appearance showed, upon cytological examination, that chromosomes quite unlike those among the normal four pairs were present. These had arisen by a fragmentation of the normal members, followed by attachments of the fragments so produced. He points out that Dobzhansky (1930) has found such alterations occurring in homozygous condition. The individuals containing them were viable and even capable of reproducing themselves.

A preliminary study of plants grown from accession number 3106 indicates that a similar situation exists in *C. syriaca*. In these individuals many types of chromosomal abnormality are to be found, including such units, appearing as supernumeraries, as are found in the cultures derived from accession number 1923. Let us assume that hybridization of the two forms of *C. alpina* has produced *syriaca*-like progeny. Either as a result of this or through some environmental agency (temperature, chemical condition of the soil, natural radiation, etc.), an alteration in chromosome affinity is brought about. Two nonhomologous chromosomes become attached in the prophase stages of cell division and, upon separation, do not retain their original form. For example, a part of one of the other chromosomes might remain attached to the satellite of the normal D chromosome. A similar effect would be produced if fragmentation preceded the attachment. Where fragments are produced which do not possess a spindle-fiber attachment, they are subsequently lost unless they become attached to a unit which possesses one. Such an attachment of a fragment would produce a chromosome with an abnormally large satellite, as in the X chromosome described earlier. If, in reality, the supernumerary has arisen by means of a modification of the normal D chromosome, this alteration would also necessarily involve a shortening of the distal arm by fragmentation or some other means. It is altogether likely that an alteration of this kind would upset the normal chromosome balance to such an extent that sterility would result. In *syriaca*, however, the X chromosome seems to have only a weak effect when present one, two, or even three times. Thus, this particular chromosome may have been introduced into otherwise normal complements and

been able to reproduce itself even in competition with other more normal types.

The fact that the extra chromosomes are reproduced in such a constant manner lends strength to the theory of chromosome individuality. Other similar examples have been used as reason for questioning its validity.

Little work has been done, to the writer's knowledge, with respect to studies of progeny from extra-chromosome plants of this type. The present study shows the tendency of certain of the higher-numbered individuals to maintain these complements. It is probable that, by continuing the work, stable races could be established.

The investigation of cytogenetic relationships of *C. alpina* and *C. syriaca* is of interest in the light of modern theories in taxonomical research. From a purely morphological consideration there is little doubt that they should be classified as distinct species. Constant differences are apparent wherever material is found, and although they do exist in the same general geographical region, their distribution is not known to overlap. Certain intermediate characters indicate that *syriaca* has arisen from *alpina* originally, but changes have occurred which make its segregation warranted.

The criteria which, cytogenetically speaking, have so far been of assistance in taxonomic treatments, are: (1) number of chromosomes, (2) chromosome size and shape, (3) compatibility of chromosomes when brought together in a hybrid, and (4) interfertility or sterility upon hybridization. As has been pointed out, the chromosomes of the two species under discussion are alike in both number and morphology with the exception of the supernumeraries present in *C. syriaca*. When crossed, a hybrid is obtained which is more or less intermediate in appearance. It shows a high degree of chromosome affinity and is as fertile as the parental species, or nearly so. Thus, with reference to chromosome affinity in the basic genom and the fertility of F_1 hybrids, the former classification, that is, treating *syriaca* as a subspecies of *C. alpina*, is probably justified. There remain, however, the supernumerary chromosomes which are unique in *syriaca* and the numerous differences in external morphology which serve to set it apart as a species.

SUMMARY

1. *Crepis syriaca* was originally described as a variety of *C. alpina* L., and unquestionably it is very closely related, but numerous constant morphological differences exist.

2. Accessions from the wild show considerable chromosomal variation involving number and morphology. Chromosome numbers are constant for the individual, but in selfed lines plants with ten, eleven, twelve,

thirteen, fourteen, fifteen, sixteen, and eighteen somatic units are found, the variation involving a small chromosome of distinctive morphology. Selfed cultures show a much greater stability of 10-chromosome lines and greater variability of lines derived from plants containing supernumeraries. This variability does not lead to the production of a majority of 10-chromosome individuals.

3. The supernumeraries have small and inconsistent effects on the external morphology of plants containing them. Abnormal flower-head development is regularly associated with the presence of two or more extra units. Ten- and 11-chromosome plants were very similar in appearance, but higher-numbered individuals varied considerably from the normal. An interesting new "dwarf" form, not associated with chromosome variation, made its appearance in 10-chromosome cultures.

4. The presence of extra chromosomes causes a marked increase in the percentage of undeveloped pollen grains produced, this increase being more or less proportional to the number of extra units. The number of supernumeraries present apparently has a greater effect in this connection than has the chromosome unbalance. There seems to be a preferential functioning in favor of gametes with more than five univalents in plants with more than ten chromosomes. Although individuals with five or more supernumeraries are entirely sterile and those with fewer are partly so, there does not seem to be a direct correlation between pollen development and fertility in these cultures.

5. At meiosis the basic genom forms five bivalents. In plants containing two or four supernumeraries complete pairing is consistently found. In 11-chromosome plants, the univalent divides at the first division. At the second, the halves go to one or the other pole and are incorporated in the daughter nuclei. Occasional nondisjunction of supernumerary units at these divisions may account for the increase in chromosome number.

6. In F_1 hybrids from *C. syriaca* $\times$ *C. alpina* the chromosome situation is closely similar to that in the pure species. So far, the extra chromosomes have been shown to be transmitted only by maternal gametes. The hybrids show complete pairing of chromosomes in 10-chromosome crosses and are highly fertile.

7. Although high fertility is shown by F_1 individuals, the percentage of undeveloped pollen increases considerably, even in 10-chromosome plants. The parental genom apparently has a greater effect on the pollen development than has that of the progeny plant. The proportion of undeveloped pollen increases with the number of extra chromosomes involved in the cross.

8. *C. rubra*, a related species, has a chromosome resembling the supernumerary of *C. syriaca*; however, the possibility that it has been involved in the origin of the latter species by hybridization is rejected on grounds of evidence from comparative morphology and geographic distribution.

9. A more acceptable hypothesis assumes the origin of *C. syriaca* through hybridization between typical *C. alpina* and a Caucasian form of *alpina* followed by chromosomal alterations in some of the hybrids, giving rise to the supernumerary chromosomes which, in this species, are unique.

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Plate 12

Crepis syriaca

- Fig. 1. Dwarf strain (ten chromosomes).
- Fig. 2. Normal plant (ten chromosomes).
- Fig. 3. 11-chromosome plant.
- Fig. 4. 12-chromosome plant.



Fig. 1



Fig. 2



Fig. 3



Fig. 4

Plate 13

Crepis syriaca

Fig. 1. 14-chromosome plant.

Crepis alpina and F₁ hybrids with *C. syriaca*

Fig. 2. *C. alpina* (ten chromosomes).

Fig. 3. 10-chromosome hybrid from cross *C. alpina* ♀ x *C. syriaca* ♂.

Fig. 4. 10-chromosome hybrid from cross *C. syriaca* ♀ x *C. alpina* ♂.



Fig. 1



Fig. 2



Fig. 3



Fig. 4

